Indian J. Phys. **70B** (5), 391–396 (1996)

IJP B
— an international journal

Particle induced X-ray emission analysis of biomedical samples : experimental set up

A K Rath, V Vijayan* and V S Ramamurthy#

Acharya Harihar Regional Centre for Cancer Research and Treatment, Cuttack-753 007, India

*Institue of Physics, Sachivalaya Marg, Bhubaneswar-751 005, India

#Department of Science & Technology, Technology Bhawan, New Mehrauli Road, New Delhi-110 016, India

Received 21 March 1996, accepted 20 June 1996

Abstract : Trace element analysis of biomedical samples using Particle Induced X-ray Emission (PIXE) technique is being done at Institute of Physics in collaboration with Regional Cancer Centre, Cuttack using a 3 MV Pelletron Particle Accelerator. A dedicated beam line with the required infrastructure has been developed. The technique, instrumentation, target irradiation, data aquisition, data analysis and the results are presented. IAEA animal blood A13 reference standard has been analysed and the standaradisation with respect to Yttirium as internal standards is presented. The results are well within acceptable limits

Keywords : PIXE, trace elements, biomedical samples

PACS Nos. : 87.64.Fb, 87.64.–t, 07 85.+n

1. Introduction

The technique of Particle Induced X-ray Emission (PIXE) since its introduction in 1970 [1] has been successfully used by various groups through out the world for trace element analysis (TEA) of biomedical samples. In India too this technique has been used for TEA [2]. But the unavailability of low energy particle accelerators and lack of interaction between the Physicists and Biologists and Medical professionals is the main reason for non popularity of PIXE. PIXE is similar to other forms of X-ray emission techniques like classical X-ray fluorescence (XRF) and electron probe microanalysis (EPMA). The difference is only in the method of excitation. In PIXE heavy charged particles, typically

Address for correspondence : A K Rath, Indraprartha Apollo Hospital, Sarita Vihar, New Delhi-110 044, India

protons of 1–4 MeV energy is used for excitation and production of characteristic X-rays from the elements present in the target. Optimum detection is achieved at around 2 MeV bombardment energy of protons due to the fact that at this energy the coulomb interaction between accelerated protons and atomic electrons of the inner shell gives maximum ionization cross section for most elements [3].

The major advantages of PIXE are its multielemental character (all elements from Na to U can in principle be measured), its high sensitivity (absolute detection limit is 10^{-12} g and relative detection limit is 0.1 μg/g), the smooth variation of the relative detection limit with atomic number of the analyte element, the ability to analyse tiny samples (1 mg or less), the speed of the analysis (1–10 min bombardment time per specimen), the possibility for automation and the fact that it is often non destructive [4]. Compared to X-rays, protons or other heavy charged particles have the advantage that they can be focussed by electrostatic or electromagnetic lenses and may be transported over large distances without loss of beam intensity. Hence compared to Energy Dispersive XRF (ED-XRF) PIXE offers detection limits which are often one order of magnitude better [5]. It allows one to analyse smaller sample masses and it is faster.

Using the 3 MV pelletron particle accelerator installaed at the Ion Beam Laboratory, Institute of Physics, Bhubaneswar an infrastructure has been developed for routine analysis of biomedical samples. Human tissues, blood and serum samples collected at regular intervals from AHRCCRT, Cuttack were prepared, irradiated and PIXE analysis was done. The results were compared with the IAEA biological standard and also compared with the published data of human trace element concentaration [6]. The results confirm that the experimental setup and the analytical procedures are acceptable. Hence routine TEA of selected biomedical samples for specific solutions can be carried out using the setup.

2. Materials and methods

2.1. Experimental setup :

The pelletron particle accelerator is a low energy (3 MV) tandem electrostatic accelerator. The beam line, developed specifically for PIXE analysis of biomedical samples is schematically shown in Figure 1. Initial experiments were carried out using the general

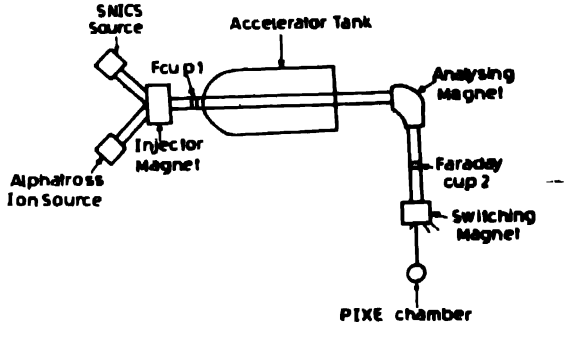

Figure 1. Schematic diagram of the experimental setup showing the accelerator.

purpose scattering chamber. For the reasons described later a special purpose PIXE hamber was designed (Figure 2). For data aquisition, a Si(Li) detector with resolution of

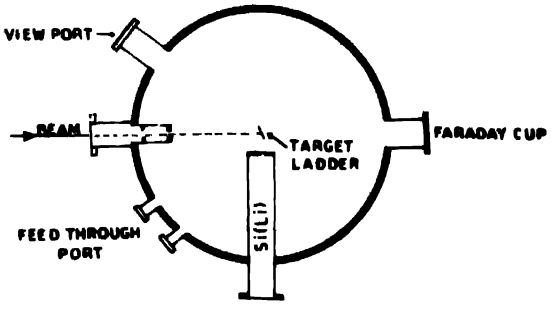

Figure 2. Cross-sectional diagram of the PIXE chamber

160 eV at 5.9 KeV, berrylium window of 8 micron thickness and a long cryostat of 22 cm length was ordered to customised specifications from Canberra Industries USA. Canberra spectroscopy amplifier, model no. 2020, is used for signal amplification. The X-ray yield, seen through the Si(Li) detector is collected using the Canberra series 88 MCA. The data is then transferred to the MICROVAX II DEC system and then to PC AT 486 ElSA computer for further analysis.

2.2. *Sample selection and preparation :*

The samples considered for study were mainly normal and pathological human tissues, whole blood and serum. The detailed procedure of sample selection, storage, processing and target preparation will be described separately in future publication. The targets are normally thin layers of the sample deposited uniformly over mylar films usually of 3 μm to 6 μm thickness. Yttrium is added as internal standard. During each run a set of IAEA animal blood reference standard A13, are prepared and analysed along with other biomedical samples under similar experimental conditions.

2.3. *Target irradiation :*

An array of samples as targets are mounted in the chamber. Till date only vacuum PIXE has been done. The chamber pressure is usually of the order of 10^{-6} torr. The proton beam of 3 MeV energy is used for routine PIXE. The target is positioned in such a way that the solid angle subtended between the target and the beam and the target and the detector is maximum and optimum. The detector is positioned such that there is no choking effect. The beam is focussed on to be centre of the target. Attempts have also been made to use diffused beam, encompassing the total target, for exact quantitative analysis. But partial target irradiation has also yielded equally accurate results due to the use of Yttrium as internal standard.

3. Results and Discussions

3.1. Data aqusisition :

The X-ray yield is seen by the Si(Li) detector after it passes through the mylar window of the chamber. Depending on the elements of interest various thicknesses of the mylar have been used as filters or absorbers placed between the mylar chamber window and the detector. This is done so to reduce the bremsstrahlung background and pile up peaks of low energy characteristic X-rays. The detector position with respect to target is critical for collecting maximum yield. However once it is set it should not be disturbed. The beam current is adjusted for maximum yield. But local heating effect damages the target and also results in charring of the samples. Current in the range of 5–10 nano amps is optimal. In case of diffused beam irradiation encompassing the whole target, the problem of beam hallow was observed. As we use Yttrium as internal standard, use of diffused beam didn't improve the overall accuracy. Hence this practice was not repeated.

3.2. Data analysis :

During the early period of analysis, IAEA AXIL software was used. But due to its limitaions in automatic quantitative analysis, the GUPIX software was obtained from Guelph University, Ontario, Canada [7]. The latest version GUPIX 95 has been used for obtaining the present set of results. The package provides nonlinear least squares fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities to elemental concentrations via a defined standardisation technique involving fundamental parameters and a predetermined instrumental constant [8]. The reference standard of IAEA, animal blood A13 has been used for quantitative estimations. Figure 3 shows a spectrum of an IAEA sample with Yttirium as internal standard. Table 1 gives the measured concentration of Br, Cu, Fe, Rb, S, Se and Zn of the IAEA reference animal blood sample, A13. The Table 1 gives the values obtained in three different runs of different sets of targets prepared during different times. The reproducibilty from run to run and also in comparison

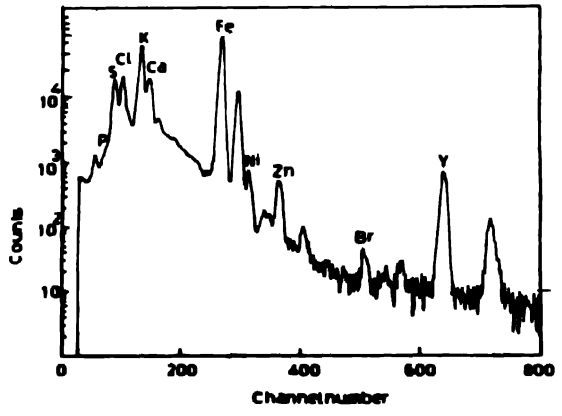

Figure 3. Spectrum of IAEA animal blood A13 reference standard showing peaks of different elements.

to the certified values is fairly good and acceptable. From the results obtained it can be observed that in case of Br, S and Se the measured concentrations are lower than that of the

Table 1. The elemental concentration of IAEA animal blood A13 reference standard in $\mu g/g$. The values given in column 2 are the certified concentrations of various elements The values in column 3, 4 and 5 are measured elemental concentrations of three different runs using diffrent sets of targets prepared at different times. Each value is average of data from 5 targets analysed in a single run. The values in the bracket are the minimum and maximum concentrations of the corresponding set of 5 targets.

Element	Certified values	First set	Second set	Third set
Br	22 (19–24)	18 (16–20)	20.0 (18.2–22.4)	17.7 (16 4–20.2)
Cu	4 3 (3.7–4.8)	4 1 (3 4–4 4)	4.2 (4.0–4 4)	4 1 (3 9–4.3)
Fe	2 4 (2.2–2.5)	2 4 (2 3–2 5)	2.4 (2 2–2 7)	2 3 (2 12–2.8)
Rb	2 3 (1.7–3.1)	2.5 (1.9–2 9)	2 4 (2.2–2.7)	2 2 (2 0–2 7)
S	6 5 (5 9–7.0)	6 3 (5.9–6.9)	6 3 (6 1–6.4)	6.1 (5 7–6 3)
Se	0.24 (0 15–0.31)	0.21 (0.13–0 27)	0 21 (0 19–0.22)	0 18 (0 17–0.19)
Zn	13 (12–14)	13.2 (12–14)	13 3 (12.7–14.2)	13.1 (12.8–13 7)

certified values. This however is consistent and can easily be attributed to the sample preparation technique followed. Due to the volatile nature of the above elements these are partially lost during acid digestion and heating. This can be corrected due the consistency of the results. Hence from the results obtained it is concluded that the system has good reproducibilty and consistency.

4. Conclusion

PIXE is a technique with higher sensitivity and lower detection limits than standard ED-XRF. The low Z matrix and availabilty of limited amount of sample makes PIXE a strong analytical tool for analysis of biomedical samples. The setup established at IOP, Bhubaneswar in collaboration with AHRCCRT, Cuttact is reliable. This can be used to obtain high quality analytical results for study of complex diagnostic and therapeutic biomedical problems relating to trace element analysis.

Acknowledgments

This work is done as a part of the BRNS project No. 4/8/93-G/740. The authors are thankful to BRNS, Department of Atomic Energy, India for the same. The authors thankfully acknowledge the help and cooperation rendered by the team at Ion Beam Laboratory, Institute of Physics, Bhubaneswar.

References

[1] T B Johansson, K R Akselsson and S A E Johansson *Nucl. Instrum. Meth.* **84** 141 (1970)

[2] Madan Lal, Daisy Joesph, R K Vajpayee, Indu Gauba, M R Lokeshwar and C S Wagle *The Science of Total Environment* **103** 209 (1991)

[3] R Boro and S Cipolla *Nucl. Instrum. Meth.* **131** 343 (1975)

[4] Willy Maenhaut *Scanning Microscopy* 4 143 (1990)

[5] W Maenhaut and Klas G Malmqvist *Hand Book of X-ray Spectrometry* (eds. Rene Van Grieken and Andrzej A Markowicz, New York : Marcel Dekker) Ch.11 p 517 (1992)

[6] G Venkatesh Iyengar *Elemental Analysis of Biological Systems* (Florida : CRC Press) Vol 1 (1989)

[7] J A Maxwell, J L Campbell and W J Teesdale *Nucl. Instrum. Meth.* **B43** 218 (1989)

[8] J A Maxwell, W J Teesdale and J L Campbell *Nucl. Instrum. Meth.* **B95** 407 (1995)

[9] J A Maxwell, W J Teesdale and J L Campbell *Nucl. Instrum. Meth.* **B95** 407 (1995)